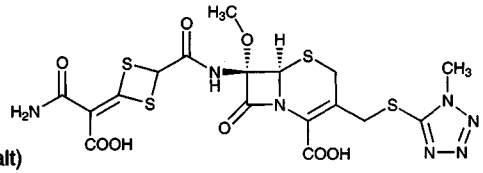
Cefotetan



Molecular formula: $C_{17}H_{17}N_7O_8S_4$
Molecular weight: 575.63
CAS Registry No.: 69712-56-7, 74356-00-6 (disodium salt)
Merck Index: 1984
Lednicer No.: 4 191, 192

SAMPLE

Matrix: blister fluid, blood
Sample preparation: Serum. 0.5 mL Serum + 2 mL MeCN, vortex for 1 min, centrifuge at 3000 g for 5 min. Remove the supernatant and add it to to 5 mL dichloromethane, vortex, centrifuge at 3000 g for 5 min, inject an aliquot of the upper layer. Blister fluid. Inject an aliquot directly.

HPLC VARIABLES

Column: μBondapak C18
Mobile phase: 100 mM phosphate buffer adjusted to pH 3.0 (?, no organic component reported)
Flow rate: 2
Detector: UV 229

CHROMATOGRAM

Retention time: 6
Limit of detection: 300 ng/mL

KEY WORDS

serum

REFERENCE

Jaresko,G.S.; Barriere,S.L.; Johnson,B.L.,Jr. Serum and blister fluid pharmacokinetics and bactericidal activities of ampicillin-sulbactam, cefotetan, cefoxitin, ceftizoxime, and ticarcillin-clavulanate [published erratum appears in Antimicrob Agents Chemother 1993 Mar;37(3):618], *Antimicrob.Agents Chemother.*, **1992**, *36*, 2233–2238.

SAMPLE

Matrix: blood, urine
Sample preparation: 200 μL Plasma + 200 μL 100 mM NaH_2PO_4 + 400 μL MeCN, mix for 5 s, let stand at 4° for 15 min, centrifuge at 10500 g for 1 min. Remove the supernatant and add it to 2 mL dichloromethane, mix for 5 min, centrifuge at 4800 g for 10 min, inject a 5-50 μL aliquot of the upper aqueous phase. Urine. Centrifuge urine at 4800 g for 10 min, dilute 1:10 with 50 mM NaH_2PO_4, inject an aliquot.

HPLC VARIABLES

Column: 125 × 4 5 μm LiChrosorb RP-18
Mobile phase: MeCN:water 7.5:92.5 containing 5.50 g/L $NaH_2PO_4.H_2O$, 1.80 g/L $Na_2HPO_4.2H_2O$, and 20 mg/L tetrabutylammonium bromide, pH 6.4 (plasma) or MeCN: water 5:95 containing 5.50 g/L $NaH_2PO_4.H_2O$, 1.80 g/L $Na_2HPO_4.2H_2O$, and 22.5 mg/L tetrabutylammonium bromide, pH 6.4 (urine)
Flow rate: 1
Injection volume: 5-50
Detector: UV 280

CHROMATOGRAM

Retention time: 6.36 (plasma, epimer A), 6.84 (plasma, epimer B), 13.09 (urine, epimer A), 14.40 (urine, epimer B)

Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Extracted: iothalamic acid (UV 254)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Kees,F.; Grobecker,H.; Naber,K.G. High-performance liquid chromatographic analysis of cefotetan epimers in human plasma and urine, *J.Chromatogr.*, **1984**, *305*, 363–371.

SAMPLE
Matrix: blood, urine
Sample preparation: Precipitate proteins with MeCN.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:70 mM sodium acetate 44:56, pH adjusted to 5.7 with acetic acid
Flow rate: 1
Detector: UV 270

CHROMATOGRAM
Internal standard: cefotetan
Limit of detection: 600 ng/mL

OTHER SUBSTANCES
Simultaneous: ceftriaxone

KEY WORDS
plasma; cefotetan is IS

REFERENCE
Paradis,D.; Vallée,F.; Allard,S.; Bisson,C.; Daviau,N.; Drapeau,C.; Auger,F.; LeBel,M. Comparative study of pharmacokinetics and serum bactericidal activities of cefpirome, ceftazidime, ceftriaxone, imipenem, and ciprofloxacin, *Antimicrob.Agents Chemother.*, **1992**, *36*, 2085–2092.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 8 4 μm Radial-Pak NOVA C18
Mobile phase: MeCN:water:acetic acid 20:80:2 containing 5 mM sodium 1 -hexanesulfonate
Flow rate: 2
Injection volume: 20
Detector: UV 264

CHROMATOGRAM
Retention time: 2.6
Internal standard: 3,5-dinitrobenzoic acid (10.2)

OTHER SUBSTANCES
Simultaneous: cefmetazole, cefodizime, cefoperazone, cefotaxime, piperacillin

REFERENCE
Marunaka,T.; Matsushima,E.; Maniwa,M. Determination of cefodizime in biological materials by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *420*, 329–339.

SAMPLE

Matrix: solutions

Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

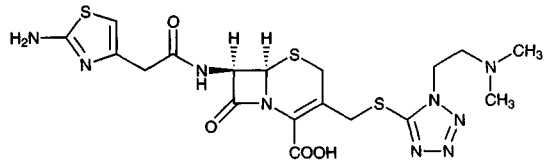
HPLC VARIABLES

Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate + 10 mM tetrabutylammonium bromide + 1% acetic acid 30:70
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 290

REFERENCE

Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

Cefotiam



Molecular formula: $C_{18}H_{23}N_9O_4S_3$
Molecular weight: 525.64
CAS Registry No.: 61622-34-2, 66309-69-1 (HCl)
Merck Index: 1985
Lednicer No.: 3 215

SAMPLE

Matrix: blister fluid, blood
Sample preparation: 200 µL Serum or blister fluid + 200 µL 50 mM pH 6.2 sodium phosphate + 400 µL MeCN, mix, add 2 mL dichloromethane, extract, inject a 50 µL aliquot of the aqueous supernatant. Refrigerate samples before injection.

HPLC VARIABLES

Column: 125 × 4.6 5 µm Spherisorb ODS-2
Mobile phase: MeCN:water:acetic acid 80:1000:2, adjust pH to 5.1 with 10 M NaOH
Column temperature: 30
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 5.4
Limit of detection: 20 ng/mL (serum), 10 ng/mL (blister fluid)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

serum

REFERENCE

Kees,F.; Raasch,W.; Steger,M.; Grobecker,H. High-performance liquid chromatographic assay for cefotiam and d3-cefotiam in human serum, *J.Chromatogr.*, **1990**, *525*, 484–489.

SAMPLE

Matrix: blood
Sample preparation: 500 µL Plasma + 100 µL 1 mg/mL cefotaxime in water + 1 mL MeCN, vortex for 3 s, centrifuge for 5 min. Remove the upper layer and add it to 3 mL dichloromethane, shake for 5 min, centrifuge, inject a 20 µL aliquot of the aqueous phase.

HPLC VARIABLES

Column: 250 × 4.6 7 µm Eicompak MA-ODS (Eicom Corp.)
Mobile phase: MeCN:10 mM pH 4.2 acetate buffer 15:85
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 2.3
Internal standard: cefotaxime (4.1)
Limit of quantitation: 100 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Chiba,K.; Tsuchiya,M.; Kato,J.; Ochi,K.; Kawa,Z.; Ishizaki,T. Cefotiam disposition in markedly obese athlete patients, Japanese sumo wrestlers, *Antimicrob.Agents Chemother.*, **1989**, *33*, 1188–1192.

SAMPLE
Matrix: blood
Sample preparation: Dilute plasma 1:5 with 50 mM pH 7.7 phosphate buffer, inject a 500 μL aliquot onto column A with mobile phase A, after 10 min backflush the contents of column A onto column B with mobile phase C, after 5 min remove column A from the circuit and continue to elute column B with mobile phase C, monitor the effluent from column B. Wash column A with mobile phase B for 10 min then re-equilibrate column A with mobile phase A for 10 min.

HPLC VARIABLES
Column: A Guard Pak μBondapak C18; B 150 × 4.6 5 μm YMC ODS A-302 (Yamamura Chemical)
Mobile phase: A 50 mM pH 7.7 phosphate buffer; B MeCN:water 60:40; C MeCN:50 mM pH 7.7 phosphate buffer 12:88
Column temperature: 25
Flow rate: 1
Injection volume: 500
Detector: UV 254

CHROMATOGRAM
Retention time: 18
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; column-switching

REFERENCE
Yamashita,K.; Motohashi,M.; Yashiki,T. Automated high-performance liquid chromatographic method for the simultaneous determination of cefotiam and delta3-cefotiam in human plasma using column switching, *J.Chromatogr.*, **1992**, *577*, 174–179.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Serum or urine + 1 mL 10 (serum) or 100 (urine) μg/mL ceftezole in MeOH, shake on a microthermomixer for 30 s, filter (urine samples only), centrifuge at 3000 rpm for 3 min, filter (0.5 μm) the supernatant, dilute with two volumes of water, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4 Nucleosil 5C18
Column: 150 × 4 Nucleosil 5C18
Mobile phase: MeCN:100 mM pH 4.4 acetate buffer 5:95 (Buffer was 100 mM acetic acid: 100 mM sodium acetate 2:1.)
Flow rate: 0.8
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 8
Internal standard: ceftezole (11)
Limit of detection: 2000 ng/mL (urine), 100 ng/mL (serum)

KEY WORDS
serum

REFERENCE
Itakura,K.; Mitani,M.; Aoki,I.; Usui,Y. High performance liquid chromatographic assay of cefsulodin, cefotiam and cefmenoxime in serum and urine, *Chem.Pharm.Bull.(Tokyo)*, **1982**, *30*, 622–627.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 200 μL Plasma + 20 μL 0.45 N phosphoric acid + 100 μL methanol + 20 μL 270 μmol/L cephalexin, vortex 15 s, centrifuge for 3 min, remove 100 μL supernatant, inject 20 μL. Urine. 10 μL Urine + 0.5 mL water + 20 μL 270 μmol/L cephalexin, vortex 15 s, remove 100 μL supernatant, inject 20 μL.

HPLC VARIABLES
Guard column: 100 × 4.7 Co:Pell ODS
Column: 150 × 4.7 LiChrosorb RP-18
Mobile phase: MeOH: 20 mM tetrabutylammonium hydrogen sulfate and 24 mM K_3PO_4 and 16 mM KH_2PO_4 23:77
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 24
Internal standard: Cephalexin
Limit of detection: 50 nmol/mL (urine), 1 nmol/mL (plasma)

OTHER SUBSTANCES
Simultaneous: cefuroxime

KEY WORDS
plasma

REFERENCE
Lecaillon,J.B.; Rouan,M.C.; Souppart,C.; Febvre,N.; Juge,F. Determination of cefsulodin, cefotiam, cephalexin, cefotaxime, deacetylcefotaxime, cefuroxime and cefroxadin in plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1982**, *228*, 257–267.

SAMPLE
Matrix: solutions
Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 15:85
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 260

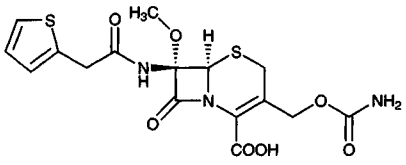
OTHER SUBSTANCES

Also analyzed: cefpiramide, cefazolin, cefmenoxime, cefbuperazone, cefoxitin, cephaloridine

REFERENCE

Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

Cefoxitin



Molecular formula: $C_{16}H_{17}N_3O_7S_2$
Molecular weight: 427.46
CAS Registry No.: 35607-66-0, 33564-30-6 (sodium salt)
Merck Index: 1986
Lednicer No.: 2 435; 4 190

SAMPLE

Matrix: blood
Sample preparation: Dilute serum with an equal volume of water, inject a 20 μL aliquot onto column A, elute column A to waste with MeOH:10 mM pH 7.0 phosphate buffer 5:95 at 0.3 mL/min, after 1.3 min elute the contents of column A onto column B with mobile phase A or B, elute with mobile phase A or B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 50 × 2.1 40 μm Supelclean LC-NH_2; B 150 × 4.6 3 μm Supelcosil LC-18
Mobile phase: A MeCN:MeOH:10 mM pH 7.0 phosphate buffer 15:20:65 containing 5 mM tetrabutylammonium hydrogen sulfate; B MeOH:10 mM pH 7.0 phosphate buffer 30:70 containing 5 mM tetrabutylammonium hydrogen sulfate
Flow rate: 1
Injection volume: 20
Detector: UV 267

CHROMATOGRAM

Retention time: 6.8 (mobile phase A), 8.0 (mobile phase B)
Limit of detection: 500-2000 ng/mL

OTHER SUBSTANCES

Extracted: cefamandole, cefazolin, cefodizime, cefoperazone, ceftizoxime, ceftriaxone, cefuroxime, cephaloridine, cephalothin
Noninterfering: acetaminophen, acyclovir, digoxin, fluconazole, ranitidine, teicoplanin, theophylline, vancomycin

KEY WORDS

column-switching; serum

REFERENCE

Bompadre,S.; Ferrante,L.; Leone,L. On-line solid-phase extraction of cephalosporins, *J.Chromatogr.A*, **1998**, *812*, 191–196.

SAMPLE

Matrix: blood
Sample preparation: Prepare an anion-exchange SPE cartridge in a 6 mL syringe barrel with a filter paper disc in the bottom. Pack with DEAE-A-25 Sephadex in PBS to a bed volume of 3 mL, wash with PBS, place filter paper on top. Add 500 μL serum to SPE cartridge, add 500 μL PBS to SPE cartridge, wash with 4 mL PBS, elute with 5 mL 1 M NaCl, inject a 100 μL aliquot of the eluate. (PBS was 8 g NaCl, 1.15 g Na_2HPO_4, 0.2 g KCl, and 0.2 g KH_2PO_4 in 1 L water, pH 7.2.)

HPLC VARIABLES

Column: 300 × 4 10 μm octadecylsilane
Mobile phase: MeCN:buffer 13:87 (Buffer was water adjusted to pH 2.8 with acetic acid, about 1.5 mL/min.)
Flow rate: 1.5

Injection volume: 100
Detector: UV 270

CHROMATOGRAM
Retention time: 7.8
Limit of quantitation: 1000 ng/mL

OTHER SUBSTANCES
Extracted: cephapirin
Noninterfering: amikacin, amphotericin B, azathioprine, carbenicillin, chloral hydrate, cimetidine, dopamine, fluphenazine, furosemide, hydrochlorothiazide, insulin, levothyroxine, methylprednisolone, nitroglycerin, oxacillin, prednisone, procainamide, sulfamethoxazole, tolazamide, tolbutamide, triamterene, trimethoprim
Interfering: cefotaxime

KEY WORDS
serum; SPE

REFERENCE
Fasching,C.E.; Peterson,L.R. Anion-exchange extraction of cephapirin, cefotaxime, and cefoxitin from serum for liquid chromatography, *Antimicrob.Agents Chemother.*, **1982**, *21*, 628–633.

SAMPLE
Matrix: blood
Sample preparation: Mix serum with an equal volume of 250 μg/mL 4'-nitroacetanilide in MeCN:MeOH 90:10, mix, let stand at room temperature for 10 min, mix, centrifuge at 12800 g for 2 min, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: RCSS Guard-Pak (Waters)
Column: 100 × 8 C18 Radial Pak (Waters)
Mobile phase: MeOH:0.75% acetic acid 30:70, pH adjusted to 5.5 with triethylamine
Flow rate: 3
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 4.2
Internal standard: 4'-nitroacetanilide (12.4)
Limit of detection: 3 μg/mL

OTHER SUBSTANCES
Extracted: cefamandole, cefazolin, cefotaxime, cephapirin, chloramphenicol
Simultaneous: acetaminophen, N-acetylprocainamide, cefaclor, cephalexin, cephalothin, cimetidine, miconazole, moxalactam, procainamide, sulfamethoxazole, theophylline, tobramycin, vancomycin

KEY WORDS
serum

REFERENCE
Danzer,L.A. Liquid-chromatographic determination of cephalosporins and chloramphenicol in serum, *Clin.Chem.*, **1983**, *29*, 856–858.

SAMPLE
Matrix: blood

Sample preparation: 300 μL Plasma + 300 μL IS in ice-cold MeOH:100 mM pH 5.2 sodium acetate 70:30, vortex for 30 s, let stand at -20° for 10 min, centrifuge at 1500 g for 10 min, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 10 μm C18
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:100 mM sodium acetate 11.52:0.48:88, pH 5.2
Flow rate: 2.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 5
Internal standard: cefoperazone (7.5)
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: cefotaxime
Interfering: cephaloridine, cephalexin

KEY WORDS

plasma

REFERENCE

Signs,S.A.; File,T.M.; Tan,J.S. High-pressure liquid chromatographic method for analysis of cephalosporins, *Antimicrob.Agents Chemother.*, **1984**, *26*, 652–655.

SAMPLE

Matrix: blood
Sample preparation: 350 μL Serum + 150 μL 150 μg/mL temocillin in water + 250 μL 400 mM HCl + 3.5 mL chloroform:n-amyl alcohol (3:1), mix for 5 min, centrifuge for 5 min. Remove the organic layer and add it to 350 μL 10 mM pH 7.0 phosphate buffer, mix for 5 min, centrifuge for 5 min, inject a 20 μL aliquot of the upper aqueous layer.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:buffer 15:85 (Buffer was 100 mM ammonium acetate adjusted to pH 4.0 with glacial acetic acid.)
Flow rate: 1.8
Injection volume: 20
Detector: UV 242

CHROMATOGRAM

Retention time: 7.4
Internal standard: temocillin (5.4)

OTHER SUBSTANCES

Extracted: cefuroxime, cephalothin, ticarcillin
Noninterfering: acetaminophen, acetazolamide, allopurinol, amikacin, ampicillin, azlocillin, caffeine, cefamandole, cefoperazone, cefotaxime, cefsulodin, ceftazidime, ceftizoxime, chloramphenicol, chlorpromazine, clindamycin, dicloxacillin, 5-fluorocytosine, flurazepam, gentamicin, methicillin, metronidazole, mezlocillin, moxalactam, nafcillin, penicillin, phenobarbital, piperacillin, procainamide, rifampin, sulfamethoxazole, theophylline, thienamycin, tobramycin, trimethoprim, vancomycin

KEY WORDS

serum

REFERENCE
Shull,V.H.; Dick,J.D. Determination of ticarcillin levels in serum by high-pressure liquid chromatography, *Antimicrob.Agents Chemother.*, **1985**, *28*, 597–600.

SAMPLE
Matrix: blood
Sample preparation: Mix 100 μL plasma + 300 μL 5 μg/mL cefotaxime in pH 3.5 10 mM acetate buffer and keep at 4°. Inject 100 μL onto column A with mobile phase A. After 5 min backflush column A with mobile phase B onto column B for 3 min. Re-equilibrate column A with mobile phase A for 16 min.

HPLC VARIABLES
Column: A 40 × 2 37-50 μm Corasil RP C18; B 20 × 4 25-40 μm Lichrosorb RP-8 + 250 × 4 Partisil ODS-3
Mobile phase: A 10 mM pH 3.5 acetate buffer; B MeCN:20 mM pH 4.3 acetate buffer 15:85
Flow rate: 1
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 12.2
Internal standard: cefotaxime
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Simultaneous: cephalexin, cefuroxime, cephaloridine
Noninterfering: cefotiam, cefadroxil, cefazolin, cefoperazone, cephalothin, cefamandole, aspirin, diclofenac, alclofenac, lonazolac, piroxicam, ibuprofen, indomethacin, ketoprofen, naproxen, phenylbutazone, mefenamic acid, caffeine

KEY WORDS
plasma; column-switching; rat; human

REFERENCE
Lee,Y.J.; Lee,H.S. Simultaneous determination of cefoxitin, cefuroxime, cephalexin and cephaloridine in plasma using HPLC and a column-switching technique, *Chromatographia*, **1990**, *30*, 80–84.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood. Add 25 μL 25 μg/mL cephalothin to 500 μL serum, vortex, place sample in an ultrafree MC filter unit with a 10000 MW cutoff (Millipore), centrifuge at 16000 g for 30 min, inject a 180 μL aliquot of the filtrate. Tissue. Homogenize 100 mg tissue in 1 mL water using a Polytron homogenizer (Brinkman), add 25 μL 25 μg/mL cephalothin, vortex, centrifuge at 1000 g for 15 min, filter (Acrodisc CR PTFE 0.2 μm filter, prewet with water and MeOH) the supernatant, inject a 180 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: μBondapak C18 Guard-Pak
Column: 100 × 8 10 μm μBondapak C18
Mobile phase: MeCN:5 mM KH_2PO_4:glacial acetic acid 22:77.5:0.5
Flow rate: 2
Injection volume: 180
Detector: UV 235

CHROMATOGRAM
Retention time: 8.75 (serum), 8.55 (tissue)

Internal standard: cephalothin (13.55 (serum), 13.95 (tissue))
Limit of detection: 10 ng/mL (serum), 50 ng/mL (tissue)

KEY WORDS
cat; colon; serum

REFERENCE
Cox,S.K.; Burnette,J.D.; Huss,B.T.; Frazier,D. Determination of cefoxitin in serum and tissue, *J.Chromatogr.B*, **1998**, *705*, 145–148.

SAMPLE
Matrix: blood, tissue
Sample preparation: Homogenize 1 g tissue with 10 mL pH 6.0 100 mM phosphate buffer (Polytron homogenizer), centrifuge at 3000 g for 10 min. 0.5 mL Serum or tissue homogenate supernatant + 2 mL MeCN, vortex for 1 min, centrifuge at 3000 g for 5 min. Remove the supernatant and add it to 5 mL dichloromethane, vortex , centrifuge at 3000 g for 5 min, inject a 50 μL aliquot of the upper layer.

HPLC VARIABLES
Column: 300 mm long μBondapak C18
Mobile phase: MeCN:100 mM sodium phosphate 14:86, adjust pH to 6.0
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 5.5

OTHER SUBSTANCES
Also analyzed: cefoperazone

KEY WORDS
serum

REFERENCE
Bawdon,R.E.; Hemsell,D.L.; Guss,S.P. Comparison of cefoperazone and cefoxitin concentrations in serum and pelvic tissue of abdominal hysterectomy patients, *Antimicrob.Agents Chemother.*, **1982**, *22*, 999–1003.

SAMPLE
Matrix: blood, urine
Sample preparation: Blood. Add 500 μL MeOH to 500 μL serum, vortex for 30 s, centrifuge at 10000 g for 15 min, inject a 50 μL aliquot of the supernatant. Urine. Add 4.5 mL 50 mM pH 6.0 potassium phosphate buffer to 500 μL urine, mix vigorously for 15 s, filter 1 mL of this solution (0.45 μm filter, Millex HA, Millipore), inject a 50 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm Nucleosil-5 C18
Column: 150 × 4.6 5 μm Nucleosil-5 C18
Mobile phase: A MeCN:100 mM pH 6.0 potassium phosphate buffer 13:87; B MeCN:100 mM pH 6.0 potassium phosphate buffer 20:80 containing 0.1 mM hexadecyltrimethylammonium
Flow rate: 1
Injection volume: 50
Detector: UV 265

CHROMATOGRAM
Retention time: 8.5 (A), 8.0 (B)
Limit of quantitation: 200 ng/mL (serum), 2 μg/mL (urine)

KEY WORDS
serum

REFERENCE
García-Glez,J.C.; Méndez,R.; Martín-Villacorta,J. Quantitative determination of semisynthetic cephamycins in human serum and urine by ion-exchange, reversed-phase and ion-pair chromatography, *J.Chromatogr.A*, **1998**, *812*, 197–204.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Dissolve in water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:acetic acid 30:70:0.1
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 12
Limit of quantitation: 1650 ng/mL

OTHER SUBSTANCES
Simultaneous: impurities, cefadroxil, cephapirin, ceftizoxime, cefaclor, cefotaxime, cephalexin, cefazolin, cephradine, cefoperazone, cefamandole, cephalothin, cefamandole nafate

REFERENCE
Ting,S. Reverse-phase liquid chromatographic analysis of cephalosporins, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1123–1130.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Nova Pak C18
Mobile phase: MeOH:5 mM pH 7.5 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 2.0

OTHER SUBSTANCES
Simultaneous: degradation products, cefazolin

KEY WORDS
injections; water; stability-indicating

REFERENCE
Stiles,M.L.; Tu,Y.H.; Allen,L.V.,Jr. Stability of cefazolin sodium, cefoxitin sodium, ceftazidime, and penicillin G sodium in portable pump reservoirs, *Am.J.Hosp.Pharm.*, **1989**, *46*, 1408–1412.

SAMPLE
Matrix: formulations
Sample preparation: Mix an aliquot with an equal volume of 5 mg/mL cefoxitin, dilute with water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Resolv (Waters)
Mobile phase: MeCN:buffer 18:86 (Buffer was 2.46 g anhydrous sodium acetate, 8 mL glacial acetic acid, and 200 mg tetrabutylammonium hydrogen sulfate in 1 L water, pH 3.0.)
Flow rate: 1.2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 3.0
Internal standard: cefoxitin

OTHER SUBSTANCES
Simultaneous: cefotaxime, metronidazole

KEY WORDS
injections; saline; cefoxitin is IS

REFERENCE
Belliveau,P.P.; Nightingale,C.H.; Quintiliani,R. Stability of cefotaxime sodium and metronidazole in 0.9% sodium chloride injection or in ready-to-use metronidazole bags, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 1561–1563.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:50 mM KH_2PO_4 7:93
Flow rate: 3
Detector: UV 273

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: cefazolin

REFERENCE
Allababidi,S.; Shah,J.C. Efficacy and pharmacokinetics of site-specific cefazolin delivery using biodegradable implants in the prevention of post-operative wound infections, *Pharm.Res.*, **1998**, *15*, 325–333.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 25 μL aliquot.

HPLC VARIABLES
Column: 100 × 4 5 μm ODS-Hypersil
Mobile phase: MeCN:10 mM ammonium acetate 10:90

Flow rate: 2
Injection volume: 25
Detector: UV 280

REFERENCE

Eley,A.; Greenwood,D. Beta-lactamases of type culture strains of the *Bacteroides fragilis* group and of strains that hydrolse cefoxitin, latamoxef and imipenem, *J.Med.Microbiol.*, **1986**, *21*, 49–57.

SAMPLE

Matrix: solutions
Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 15:85
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 240

OTHER SUBSTANCES

Also analyzed: cefpiramide, cefazolin, cefmenoxime, cefbuperazone, cefotiam, cephaloridine

REFERENCE

Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 125 × 4.6 Lichrospher 100 RP-18
Mobile phase: MeOH:2.5 mM pH 5.6 sodium phosphate buffer 18:80
Flow rate: 1
Injection volume: 20
Detector: UV 274

CHROMATOGRAM

Retention time: 2.5
Limit of detection: 60 nM

OTHER SUBSTANCES

Simultaneous: cefoperazone, ceftazidime, cefuroxime, cephalexin, cephradine

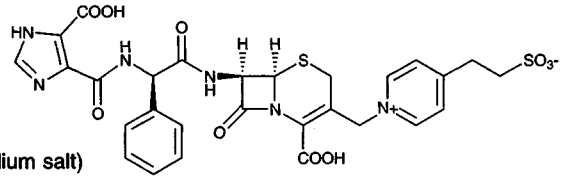
KEY WORDS

comparison with capillary electrophoresis

REFERENCE

Choi,O.-K.; Song,Y.-S. Determination of cefuroxim levels in human serum by micellar electrokinetic capillary chromatography with direct sample injection, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1265–1270.

Cefpimizole



Molecular formula: $C_{28}H_{26}N_6O_{10}S_2$
Molecular weight: 670.68
CAS Registry No.: 84880-03-5, 85287-61-2 (sodium salt)
Merck Index: 1988
Lednicer No.: 4 185

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 1 mL buffer + 4 mL MeCN, mix, let stand at least 2 h at 4°, centrifuge, remove the supernatant, wash the precipitate with 2 mL MeCN:buffer 75:25, centrifuge. Combine the supernatants and add them to 200 μL dichloromethane, let stand at -20°. Remove the aqueous phase and add it to 50 μL 200 μg/mL acetophenone in MeOH, mix, filter (0.45 μm, Gelman Acrodisc-CR), store at 4°, inject an aliquot. Urine. 100 μL Urine + 4 mL 10 μg/mL acetophenone in mobile phase, mix, filter (0.45 μm, Gelman Acrodisc-CR), store at 4°, inject an aliquot. (Buffer was 10 mM EDTA and 50 mM tetrabutylammonium hydroxide, pH 5.0.)

HPLC VARIABLES

Guard column: 50 × 2.1 35 μm Co:Pell ODS
Column: 250 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeOH:buffer 140:260, pH adjusted to 6.0 with glacial acetic acid (Buffer was 40 mL 100 mM EDTA, 50 mL 400 mM tetrabutylammonium hydroxide, and 2510 mL water.)
Injection volume: 100
Detector: UV 254

CHROMATOGRAM

Retention time: 12
Internal standard: acetophenone (18)
Limit of detection: 50 ng/mL (plasma), 1000 ng/mL (urine)
Limit of quantitation: 330 ng/mL (plasma), 16800 ng/mL (urine)

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Lakings,D.B.; Wozniak,J.M. High-performance liquid chromatographic methods for the determination of cefpimizole in plasma and urine, *J.Chromatogr.*, **1984**, *308*, 261–271.

SAMPLE

Matrix: tissue

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 3 mL MeCN and 3 mL buffer. Homogenize 250 mg rat tissue with 2.5 mL water and 0.5 mL 100 mM EDTA in a small tissue grinder (Ace Glass), add a 100 μL aliquot to the SPE cartridge, wash with 3 mL water, wash with 2 mL MeCN:water 10:90, elute with 2 mL MeCN:water 30:70, add 10 μg acetophenone to the eluate, mix, filter (0.45 μm, Gelman Acrodisc CR), store at 4°, inject an aliquot. (Buffer was 10 mM EDTA and 50 mM tetrabutylammonium hydroxide, pH 5.)

HPLC VARIABLES

Guard column: 50 × 2.1 35 μm Co:Pell ODS
Column: 250 × 4.5 5 μm C18 (IBM)

Mobile phase: MeOH:water 33:67 containing 1 mM EDTA and 5 mM tetrabutylammonium hydroxide, adjusted to pH 6.5 with acetic acid
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 14
Internal standard: acetophenone (21)
Limit of detection: 120000 ng/g

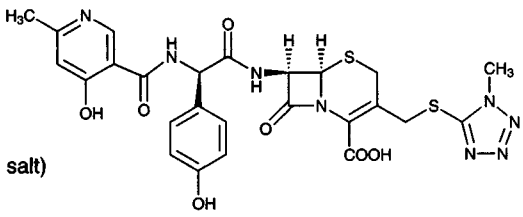
KEY WORDS

rat; liver; spleen; kidney; SPE

REFERENCE

Friis,J.M.; Lakings,D.B. High-performance liquid chromatographic method for the determination of cefpimizole in tissue, *J.Chromatogr.*, **1986**, *382*, 399–404.

Cefpiramide



Molecular formula: $C_{25}H_{24}N_8O_7S_2$
Molecular weight: 612.65
CAS Registry No.: 70797-11-4, 74849-93-7 (sodium salt)
Merck Index: 1989
Lednicer No.: 4 188

SAMPLE

Matrix: bile, blood, duodenal fluid, tissue, urine
Sample preparation: Serum, bile, duodenal fluid. 500 μL Serum, bile or duodenal fluid + 500 μL MeCN, centrifuge. Remove the supernatant and add it to 3.5 mL dichloromethane, centrifuge, inject a 20 μL aliquot of the supernatant. Urine. Dilute urine 1:10 with MeOH, inject an aliquot. Tissue. Wash gallbladder wall with pH 7 phosphate buffer, dry, weigh, homogenize in 1 mL pH 7 phosphate buffer, filter, microfilter, inject an aliquot of the microfiltrate.

HPLC VARIABLES

Column: reverse phase
Mobile phase: MeCN:water:200 mM ammonium acetate 15:75:10, adjust pH to 5.0 with glacial acetic acid
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Limit of detection: 1000 ng/mL (urine), 500 ng/mL (bile), 50 ng/mL (serum, duodenal juice)

KEY WORDS

serum; gallbladder wall; pharmacokinetics

REFERENCE

Brogard,J.M.; Jehl,F.; Adloff,M.; Blickle,J.F.; Monteil,H. High hepatic excretion in humans of cefpiramide, a new cephalosporin, *Antimicrob.Agents Chemother.*, **1988**, *32*, 1360–1364.

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Dilute urine and bile with water as necessary. 50 μL Plasma, diluted urine, or diluted bile + 50 μL 10% perchloric acid + 50 μL 3-butylxanthine in phosphate buffer, mix, centrifuge at 11000 g for 10 min. Add the supernatant to 240 μL 1 M sodium acetate, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Cosmosil 5C18 (Nacalai Tesque)
Mobile phase: MeOH:30 mM pH 5.0 isotonic phosphate buffer 20:80
Column temperature: 40
Flow rate: 1.5
Detector: UV 279

CHROMATOGRAM

Internal standard: 3-butylxanthine

KEY WORDS

plasma; rat; pharmacokinetics

REFERENCE
Muraoka,I.; Hasegawa,T.; Nadai,M.; Wang,L.; Haghgoo,S.; Tagaya,O.; Nabeshima,T. Biliary and renal excretions of cefpiramide in Eisai hyperbilirubinemic rats, *Antimicrob.Agents Chemother.*, **1995**, *39*, 70–74.

SAMPLE
Matrix: blood
Sample preparation: Filter plasma (0.22 μm), inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm GFF-S5-80 internal-surface reversed phase "Pinkerton" (Regis)
Mobile phase: THF:100 mM potassium phosphate 2.5:97.5, pH 7.0
Flow rate: 1
Injection volume: 10
Detector: UV 270

CHROMATOGRAM
Retention time: 8.4

KEY WORDS
plasma; direct injection

REFERENCE
Nakagawa,T.; Shibukawa,A.; Shimono,N.; Kawashima,T.; Tanaka,H.; Haginaka,J. Retention properties of internal-surface reversed-phase silica packing and recovery of drugs from human plasma, *J.Chromatogr.*, **1987**, *420*, 297–311.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. 300 μL Serum + 300 μL cold (iced) 38.5 μg/mL cefamandole in MeOH:100 mM pH 5.2 sodium acetate 80:20, vortex for 30 s, let stand at -20° for 10 min, centrifuge at 1500 g for 10 min, inject a 10 μL aliquot. Urine. 100 μL Urine + 1 mL 76.9 μg/mL cefamandole in MeOH, vortex for 30 s, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm C18 (Waters)
Mobile phase: MeCN:MeOH:100 mM sodium acetate:water 14.4:0.6:10:75, pH 5.2
Flow rate: 2
Injection volume: 5-10
Detector: UV 254

CHROMATOGRAM
Retention time: 6.5
Internal standard: cefamandole (9.5)
Limit of detection: 12850 ng/mL (urine), 920 ng/mL (serum)

OTHER SUBSTANCES
Noninterfering: metronidazole, vancomycin, nafcillin, ticarcillin, clindamycin, gentamicin

KEY WORDS
serum

REFERENCE
Conte,J.E.,Jr.; Zurlinden,E. Column liquid chromatographic determination of cefpiramide in human serum and urine, *J.Chromatogr.*, **1987**, *417*, 452–457.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 100 μL Plasma + 100 μL 10 μg/mL cefoperazone in 100 mM pH 5 ammonium acetate, vortex for 15 s, centrifuge at 8700 g for 2 min, inject a 20-200 μL aliquot onto column A with mobile phase A, after 2 min backflush the contents of column A onto column B with mobile phase B, elute column B with mobile phase B and monitor the effluent. Re-equilibrate column A with mobile phase A before the next injection.. Urine. Dilute urine with saline. 100 μL Diluted urine + 500 μL 10 μg/mL cefoperazone in 100 mM pH 5 ammonium acetate, inject a 20-200 μL aliquot onto column A with mobile phase A, after 2 min backflush the contents of column A onto column B with mobile phase B, elute column B with mobile phase B and monitor the effluent. Re-equilibrate column A with mobile phase A before the next injection.

HPLC VARIABLES

Column: A 20 × 4 38-50 μm Corasil C18; B 100 × 8 10 μm μBondapak C18

Mobile phase: A water:triethylamine 1000:4 adjusted to pH 3.0 with orthophosphoric acid; B MeCN:water:triethylamine 750:250:4, pH adjusted to 3.0 with orthophosphoric acid

Flow rate: A 2; B 3.8

Injection volume: 20-200

Detector: UV 270

CHROMATOGRAM

Retention time: 2.33

Internal standard: cefoperazone (3.66)

Limit of detection: 250 ng/mL

OTHER SUBSTANCES

Simultaneous: ceftazidime, ceftriaxone, cefotaxime, cephaloridine, ceforanide, moxalactam, cefazolin, cefonicid, cephalothin

Noninterfering: cefotiam, cefadroxil

KEY WORDS

plasma; column-switching

REFERENCE

Demotes-Mainard,F.; Vinçon,G.; Jarry,C.; Necciari,J.; Albin,H. Micromethod for the determination of cefpiramide in human plasma and urine by high-performance liquid chromatography using automated column switching, *J.Chromatogr.*, **1987**, *419*, 388–395.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 ODS-80TM (Tosoh)

Mobile phase: MeCN:buffer 20:80 (Buffer was 10 mM tetrabutylammonium bromide and 10 mM ammonium acetate.)

Detector: UV 240

REFERENCE

Tamai,I.; Maekawa,T.; Tsuji,A. Membrane potential-dependent and carrier-mediated transport of cefpiramide, a cephalosporin antibiotic, in canalicular rat liver plasma membrane vesicles, *J.Pharmacol.Exp.Ther.*, **1990**, *253*, 537–544.

SAMPLE

Matrix: solutions

Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 15:85
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 240

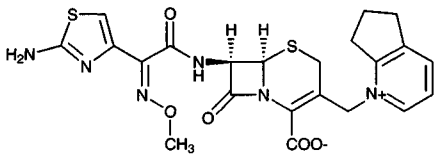
OTHER SUBSTANCES

Also analyzed: cefazolin, cefmenoxime, cefbuperazone, cefoxitin, cefotiam, cephaloridine

REFERENCE

Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

Cefpirome



Molecular formula: $C_{22}H_{22}N_6O_5S_2$
Molecular weight: 514.59
CAS Registry No.: 84957-29-9, 98753-19-6 (sulfate)
Merck Index: 1990
Lednicer No.: 5 158

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 50 μL cefpirome sulfate solution + 100 μL 10% trichloroacetic acid, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: C18
Mobile phase: MeCN:50 mM pH 3.5-4.0 ammonium phosphate buffer 8:92
Flow rate: 1.2
Injection volume: 20
Detector: UV 257

CHROMATOGRAM
Internal standard: cefpirome

OTHER SUBSTANCES
Extracted: ceftazidime

KEY WORDS
cefpirome is IS; serum

REFERENCE
Klepser,M.E.; Patel,K.B.; Nicolau,D.P.; Quintiliani,R.; Nightingale,C.H. Comparison of the bactericidal activities of ofloxacin and ciprofloxacin alone and in combination with ceftazidime and piperacillin against clinical strains of *Pseudomonas aeruginosa*, *Antimicrob.Agents Chemother.*, **1995**, *39*, 2503–2510.

SAMPLE
Matrix: blood, urine
Sample preparation: Add aminophylline, precipitate proteins with MeCN, delipidate with dichloromethane.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeOH:100 mM sodium acetate 14:86, pH adjusted to 4.2 with acetic acid
Flow rate: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 7.8
Internal standard: aminophylline
Limit of detection: 100 ng/mL

KEY WORDS
plasma

REFERENCE

Paradis,D.; Vallée,F.; Allard,S.; Bisson,C.; Daviau,N.; Drapeau,C.; Auger,F.; LeBel,M. Comparative study of pharmacokinetics and serum bactericidal activities of cefpirome, ceftazidime, ceftriaxone, imipenem, and ciprofloxacin, *Antimicrob.Agents Chemother.*, **1992**, *36*, 2085–2092.

SAMPLE

Matrix: cell suspensions
Sample preparation: Filter (0.45 μm).

HPLC VARIABLES

Column: 150 × 4.6 5 μm Ultrasphere IP ion pair
Mobile phase: MeOH:100 mM sodium perchlorate adjusted to pH 2.5 with concentrated sulfuric acid 35:65
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 3.8

OTHER SUBSTANCES

Extracted: carumonam (UV 295), ceftriaxone, cefotaxime

REFERENCE

Bellido,F.; Pechère,J.-C.; Hancock,R.E.W. Novel method for measurement of outer membrane permeability to new β-lactams in intact *Enterobacter cloacae* cells, *Antimicrob.Agents Chemother.*, **1991**, *35*, 68–72.

SAMPLE

Matrix: milk, urine
Sample preparation: Milk. 50 μL Milk + 200 μL 200 μg/mL β-hydroxypropyltheophylline in isopropanol:water 80:20, vortex for 20 s, centrifuge at 2000 g at 4° for 2 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 25°, reconstitute the residue in 200 μL mobile phase, vortex for 20 s, centrifuge at 2000 g at 4° for 2 min, inject a 10 μL aliquot. Urine. Centrifuge at 1500 g at 4° for 5 min. Remove 50 μL of the supernatant and add it to 200 μL 200 μg/mL β-hydroxypropyltheophylline in mobile phase, vortex for 15 s, inject a 10-20 μL aliquot.

HPLC VARIABLES

Column: 100 × 2.1 5 μm ODS (Hewlett-Packard)
Mobile phase: MeOH:buffer 12:88 (milk) or 10:90 (urine) (Buffer was 0.3% triethylamine in water adjusted to pH 5.1 with glacial acetic acid.)
Column temperature: 50
Flow rate: 0.5
Injection volume: 10-20
Detector: UV 240

CHROMATOGRAM

Retention time: 2.0 (milk), 2.8 (urine)
Internal standard: β-hydroxypropyltheophylline (4.0 (milk), 5.0 (urine))
Limit of detection: 625 ng/mL

OTHER SUBSTANCES

Noninterfering: ampicillin, tobramycin, gentamicin, amikacin, ticarcillin, aspirin, acetaminophen, ibuprofen, theophylline, caffeine, chlorpheniramine, cimetidine, carbamazepine, phenytoin, phenobarbital

REFERENCE

Kearns,G.L.; Johnson,V.A.; Hendry,I.R.; Wells,T.G. Microanalytical high-performance liquid chromatographic assay for cefpirome in human milk and urine, *J.Chromatogr.*, **1992**, *574*, 356–360.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 4.6 Nucleosil 10C18
Mobile phase: MeCN:1% KH_2PO_4 1:8
Flow rate: 1
Injection volume: 20
Detector: UV (wavelength not specified)

OTHER SUBSTANCES

Simultaneous: degradation products

REFERENCE

Sugioka,T.; Asano,T.; Chikaraishi,Y.; Suzuki,E.; Sano,A.; Kuriki,T.; Shirotsuka,M.; Saito,K. Stability and degradation pattern of cefpirome (HR 810) in aqueous solution, *Chem.Pharm.Bull.(Tokyo)*, **1990**, *38*, 1998–2002.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeCN:phosphate buffer 8:92
Flow rate: 1.2

OTHER SUBSTANCES

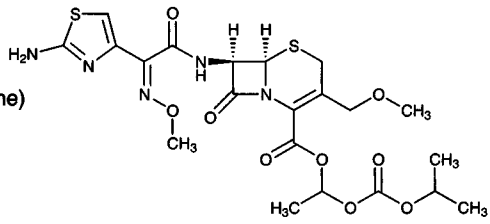
Simultaneous: ceftazidime

REFERENCE

Nicolau,D.P.; Nightingale,C.H.; Banevicius,M.A.; Fu,Q.; Quintiliani,R. Serum bactericidal activity of ceftazidime: Continuous infusion versus intermittent injections, *Antimicrob.Agents Chemother.*, **1996**, *40*, 61–64.

Cefpodoxime proxetil

Molecular formula: $C_{21}H_{27}N_5O_9S_2$
Molecular weight: 557.61
CAS Registry No.: 87239-81-4, 80210-62-4 (cefpodoxime)
Merck Index: 1991
Lednicer No.: 5 158



SAMPLE

Matrix: blood
Sample preparation: Condition a C8 SPE cartridge with 1 mL MeOH:DMF 90:10 and 1 mL 1% phosphoric acid, do not allow to go dry. 200 µL Plasma + 1 mL 1 µg/mL cefaclor in 1% phosphoric acid + 200 µL MeCN:1% phosphoric acid 1:99, add to the SPE cartridge, wash with 1 mL MeOH:1% phosphoric acid 5:95, wash with 500 µL 1% phosphoric acid, elute the contents of the SPE cartridge onto the analytical column with the mobile phase.

HPLC VARIABLES

Guard column: 12 × 4.6 7 µm Newguard C8
Column: 250 × 4.6 5 µm IB-SIL C18 (Phenomenex)
Mobile phase: MeCN:MeOH:50 mM pH 6.0 sodium acetate buffer 4:4:92 (After elution of IS inject 1 mL MeCN:water 90:10 to remove late eluting peaks.)
Flow rate: 2
Detector: UV 254

CHROMATOGRAM

Retention time: 10.8
Internal standard: cefaclor (17)
Limit of detection: 3 ng/mL
Limit of quantitation: 11 ng/mL

OTHER SUBSTANCES

Extracted: caffeine, cefotaxime
Noninterfering: acetaminophen, amikacin, ceftazidime, ceftriaxone, gentamicin, nafcillin, phenytoin, ticarcillin, tobramycin, vancomycin
Interfering: theophylline

KEY WORDS

SPE; plasma

REFERENCE

Steenwyk,R.C.; Brewer,J.E.; Royer,M.E.; Cathcart,K.S. Reversed-phase liquid chromatographic determination of cefpodoxime in human plasma, *J.Liq.Chromatogr.*, **1991**, *14*, 3641–3656.

SAMPLE

Matrix: blood, ear fluid
Sample preparation: 50 µL Plasma or ear effusion + 50 µL 40 µg/mL cefuroxime in water + 2 mL MeCN, vortex briefly, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 75 µL mobile phase, inject a 25 µL aliquot.

HPLC VARIABLES

Guard column: 10 × 2.1 5 µm Hypersil C18
Column: 250 × 2.1 5 µm Hypersil C18

Mobile phase: MeCN:buffer 7.5:92.5 After elution of IS increase ratio to 50:50 to clean column. (Buffer was 25 mM acetate and 15 mM triethylamine adjusted to pH 4.3 with NaOH.)
Column temperature: 40
Flow rate: 0.35
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 3.5
Internal standard: cefuroxime (5.9)
Limit of detection: 20 ng/mL

KEY WORDS
plasma; chinchilla; middle ear effusion; pharmacokinetics

REFERENCE
Lovdahl,M.J.; Reher,K.E.; Russlie,H.Q.; Canafax,D.M. Determination of cefpodoxime levels in chinchilla middle ear fluid and plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *653*, 227–232.

SAMPLE
Matrix: blood, sinus mucosa
Sample preparation: Plasma. Condition a 3 mL 500 mg Bond Elut C8 SPE cartridge with 3 mL MeOH and 2 mL 1% phosphoric acid. 500 μL Plasma + 1 mL 2 μg/mL cefaclor in 1% phosphoric acid, mix, add to the SPE cartridge, wash with 3 mL 1% perchloric acid, elute with 750 μL MeOH, inject a 50 μL aliquot of the eluate. Sinus mucosa. Condition a 1 mL Bond Elut C8 SPE cartridge with 1 mL MeOH and 1 mL 1% phosphoric acid. Chop sample with a scalpel, weigh out 20 mg and add it to 500 μL 10 mM pH 7.0 phosphate buffer, rotate at 4° for 12 h, centrifuge at 800 g for 10 min. 400 μL Supernatant + 1 mL 50 ng/mL cefaclor, mix, add to the SPE cartridge, wash with 1% perchloric acid, elute with 150 μL MeOH, inject a 75 μL aliquot of the eluate.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm C18 (Shandon)
Column: 250 × 4.6 5 μm Supelcosil LC 18
Mobile phase: MeOH:MeCN:50 mM pH 3.8 acetate buffer 10:3:87 (plasma) or 12:2:86 (sinus mucosa)
Flow rate: 1
Injection volume: 50-75
Detector: UV 235

CHROMATOGRAM
Retention time: 16.8
Internal standard: cefaclor (18.2)
Limit of detection: 10 ng/mL (plasma)
Limit of quantitation: 130 ng/g (sinus mucosa), 50 ng/mL (plasma)

KEY WORDS
plasma; SPE

REFERENCE
Camus,F.; Deslandes,A.; Harcouet,L.; Farinotti,R. High-performance liquid chromatographic method for the determination of cefpodoxime levels in plasma and sinus mucosa, *J.Chromatogr.B*, **1994**, *656*, 383–388.

SAMPLE
Matrix: blood, tissue, urine

Sample preparation: Serum. 500 μL Serum + 500 μL MeCN, vortex, rotate at 20 rpm for 10 min, centrifuge at 1000 g for 10 min. Remove the supernatant and add it to 3.2 mL dichloromethane, rotate at 20 rpm for 10 min, centrifuge at 1000 g for 10 min, inject a 20 μL aliquot of the upper aqueous layer. Urine. Dilute urine 1:10 with water, inject an aliquot. Tissue. Grind frozen under liquid nitrogen, extract with 3 mL pH 7 phosphate buffer at 4° for 12 h, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES

Column: 70 × 4.6 3 μm Ultrasphere XL-ODS
Mobile phase: MeCN:21.5 mM ammonium acetate 7:93, adjusted to pH 5 with glacial acetic acid
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 2.6
Limit of detection: 300 ng/mL (urine), 20 ng/mL (serum)

OTHER SUBSTANCES

Noninterfering: aminoglycosides, amoxicillin, ampicillin, aztreonam, carbamazepine, cefadroxil, cefixime, cefotaxime, cefpiramide, ceftazidime, digitoxin, furosemide, lidocaine, phenobarbital, quinidine, quinolones, salicylic acid, theophylline

KEY WORDS

serum; kidney

REFERENCE

Molina,F.; Jehl,F.; Gallion,C.; Penner,F.; Monteil,H. Determination of the third generation oral cephalosporin cefpodoxime in biological fluids by high-speed high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *563*, 205–210.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 18.628 (peak 1), 18.945 (peak 2)

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: urine
Sample preparation: Filter (0.45 μm) urine, inject a 50 μL aliquot onto column A and elute to waste with mobile phase A. After 10 min elute the contents of column A onto column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Wash column A with MeOH: water for 2 min then re-equilibrate column A with mobile phase A for 6 min.

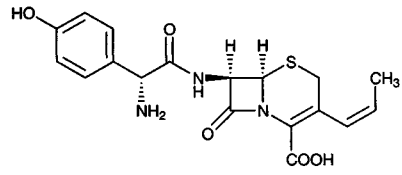
HPLC VARIABLES
Column: A 30 × 4.6 5 μm C18 (Brownlee) (Condition a new column with 30 mL MeOH and 30 mL MeOH:water 50:50.); B 250 × 4.6 5 μm IB-SIL C18 (Phenomenex)
Mobile phase: A MeOH:0.2% pH 2.0 phosphoric acid 10:90; B MeCN:50 mM pH 5.2 sodium acetate buffer 7:93
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 19
Limit of detection: 34 ng/mL
Limit of quantitation: 110 ng/mL

KEY WORDS
column-switching; pharmacokinetics

REFERENCE
Bombardt,P.A.; Cathcart,K.S.; Bothwell,B.E.; Closson,S.K. Determination of cefpodoxime levels and cefpodoxime stability in human urine by direct injection HPLC with column-switching, *J.Liq.Chromatogr.*, **1991**, *14*, 1729–1746.

Cefprozil



Molecular formula: $C_{18}H_{19}N_3O_5S$
Molecular weight: 389.43
CAS Registry No.: 92665-29-7, 121123-17-9 (monohydrate)
Merck Index: 1992
Lednicer No.: 5 158

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 100 μL water + 50 μL 25 μg/mL cefprozil + 100 μL 1 M hydrochloric acid, vortex briefly. Filter (Centrifree micropartition unit) 1 mL mixture while centrifuging at 2000 g for 10 min. Inject a 25 μL aliquot of the ultrafiltrate. Urine. 250 μL urine + 500 μL 250 μg/mL cefprozil + 4.25 mL 10 mM pH 3.5 acetate buffer, vortex briefly. Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Nucleogil C18 (plasma) or 125 × 4.6 5 μm Lichrosorb C18 (urine)
Mobile phase: MeCN:10 mM phosphoric acid (adjusted to pH 3.8 with NaOH) 8:92 (plasma) or MeCN:10 mM phosphoric acid (adjusted to pH 3.8 with NaOH) 10:90 (urine)
Flow rate: 1.2 (plasma), 1.0 (urine)
Injection volume: 25
Detector: UV 260

CHROMATOGRAM

Retention time: 9.2 (plasma), 6.3 (urine)
Internal standard: cefprozil

OTHER SUBSTANCES

Extracted: cephalexin

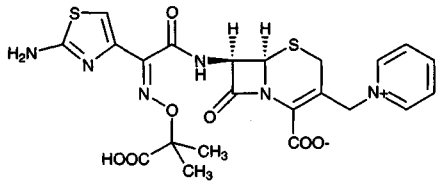
KEY WORDS

plasma; cefprozil is IS

REFERENCE

Barbhaiya,R.H. A pharmacokinetic comparison of cefadroxil and cephalexin after administration of 250, 500 and 1000 mg solution doses, *Biopharm.Drug Dispos.*, **1996**, *17*, 319–330.

Ceftazidime



Molecular formula: $C_{22}H_{22}N_6O_7S_2$
Molecular weight: 546.58
CAS Registry No.: 72558-82-8, 78439-06-2 (pentahydrate)
Merck Index: 1995
Lednicer No.: 4 192

SAMPLE

Matrix: blood, CSF
Sample preparation: Deproteinize serum or CSF with MeCN, centrifuge, add the supernatant to dichloromethane, inject a 100 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: reversed-phase
Mobile phase: MeCN:100 mM pH 5.0 NaH_2PO_4 buffer 8:92 containing 5 mM pentanesulfonic acid
Injection volume: 100
Detector: UV 254

CHROMATOGRAM

Limit of quantitation: 94 ng/mL (serum), 67 ng/mL (CSF)

KEY WORDS

serum; pharmacokinetics

REFERENCE

Nau,R.; Prange,H.W.; Kinzig,M.; Frank,A.; Dressel,A.; Scholz,P.; Kolenda,H.; Sörgel,F. Cerebrospinal fluid ceftazidime kinetics in patients with external ventriculostomies, *Antimicrob.Agents Chemother.*, **1996**, *40*, 763–766.

SAMPLE

Matrix: formulations
Sample preparation: Reconstitute ceftazidime injection in sodium carbonate with 10 mL 0.9% NaCl, agitate vigorously, dilute with 0.9% NaCl injection to obtain a ceftazidime concentration of 60 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC18
Mobile phase: MeCN:100 mM pH 7.0 K_3PO_4 buffer 6:94
Flow rate: 1
Injection volume: 20
Detector: UV 257

CHROMATOGRAM

Retention time: 8.4
Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Simultaneous: degradation products, pyridine

KEY WORDS

injections; stability-indicating

REFERENCE

Stendal,T.L.; Klem,W.; Tonnesen,H.H.; Kjonniksen,I. Drug stability and pyridine generation in ceftazidime injection stored in an elastomeric infusion device, *Am.J.Health-Syst.Pharm.*, **1998**, *55*, 683–685.

SAMPLE

Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm cyano
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 2
Injection volume: 20
Detector: UV 210

CHROMATOGRAM

Retention time: 1.73

OTHER SUBSTANCES

Simultaneous: granisetron (UV 300)

KEY WORDS

stability-indicating; injections; saline

REFERENCE

Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve sample in mobile phase to a concentration of about 1 mg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm β-CyD (Advanced Separation Technologies Inc., USA)
Mobile phase: MeOH:buffer 42:58 (Buffer was 5 mM tetraethylammonium acetate adjusted to pH 3.6 with glacial acetic acid.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 10
Detector: UV 230

CHROMATOGRAM

Retention time: 39.5

OTHER SUBSTANCES

Simultaneous: 7-ACA, 7-ADCA, cefaclor, cefaloridine, cefazolin, cefoperazone, cefotaxime, cephalosporin C

REFERENCE

Tsou,T.-L.; Wu,J.-R.; Wang,T.-M. The effects of separation of cephalosporins by HPLC with β-cyclodextrin bonded stationary phase, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1081–1095.

SAMPLE

Matrix: solutions

Sample preparation: Inject a 20 μL aliquot of an aqueous solution.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb C-6
Mobile phase: MeCN:50 mM sodium acetate containing 100 mM acetic acid 6:94 (A) or 4:96 (B)
Flow rate: 1.4
Injection volume: 20
Detector: UV 258

CHROMATOGRAM
Retention time: 5.3 (A), 7.2 (B)

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Fubara,J.O.; Notari,R.E. A kinetic oxymoron: Concentration-dependent first-order rate constants for hydrolysis of ceftazidime, *J.Pharm.Sci.*, **1998**, *87*, 53–58.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot of an aqueous solution.

HPLC VARIABLES
Column: 100 × 4.6 4 μm Novapak C18
Mobile phase: MeCN:7.5 mM pH 7.0 phosphate buffer 40:60 containing 2 g/L tetrabutylammonium phosphate
Flow rate: 1.5
Injection volume: 10
Detector: UV 242

CHROMATOGRAM
Retention time: 1.6
Internal standard: ceftriaxone

REFERENCE
Plumridge,R.J.; Rieck,A.M.; Annus,T.P.; Langton,S.R. Stability of ceftriaxone sodium in polypropylene syringes at -20, 4, and 20°C, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 2320–2323.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4.6 Lichrospher 100 RP-18
Mobile phase: MeOH:2.5 mM pH 5.6 sodium phosphate buffer 18:80
Flow rate: 1
Injection volume: 20
Detector: UV 274

CHROMATOGRAM
Retention time: 1.5
Limit of detection: 60 nM

OTHER SUBSTANCES
Simultaneous: cefoperazone, cefoxitin, cefuroxime, cephalexin, cephradine

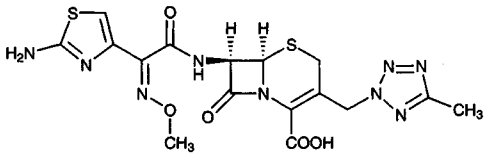
KEY WORDS

comparison with capillary electrophoresis

REFERENCE

Choi,O.-K.; Song,Y.-S. Determination of cefuroxim levels in human serum by micellar electrokinetic capillary chromatography with direct sample injection, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1265–1270.

Cefteram



Molecular formula: $C_{16}H_{17}N_9O_5S_2$
Molecular weight: 479.50
CAS Registry No.: 82547-58-8
Merck Index: 1996

SAMPLE

Matrix: blood
Sample preparation: Condition a Bond Elut phenyl SPE cartridge with 3 mL MeOH and 3 mL buffer. 1 mL Plasma + 50 μL 500 μg/mL penicillin G (sodium salt) in MeOH + 2 mL buffer, vortex, add to SPE cartridge, wash with buffer, elute with 500 μL MeOH:10 mM pH 5.2 potassium phosphate buffer 90:10, inject a 25 μL aliquot. (Buffer was 121 g Trizma base in 1 L water, adjust pH to 7.0 with concentrated HCl. Dilute 1:100 to obtain the 10 mM buffer.)

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak phenyl
Mobile phase: MeOH:10 mM pH 5.2 potassium phosphate buffer 20:80 (Buffer was 1 M KH_2PO_4 adjusted to pH 5.2 with 5 M KOH, dilute 1:100 with water to give working buffer.)
Column temperature: 50
Flow rate: 0.9
Injection volume: 25
Detector: UV 225

CHROMATOGRAM

Retention time: 4.6
Internal standard: penicillin G (12)
Limit of quantitation: 59.8 ng/mL

KEY WORDS

plasma; SPE; method stated to be applicable to urine (no details)

REFERENCE

Hicks,C.M.; Powell,M.L. Rapid analysis of ceftetrame in human plasma using sorbent extraction and high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *497*, 349–354.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 6 5 μm YMC Pack AMC312 ODS (Yamamura Chemical)
Mobile phase: MeCN:water:60% perchloric acid:sodium perchlorate monohydrate 156:844:1:5 (v/v/v/w)
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 13.6
Internal standard: cefteram

OTHER SUBSTANCES

Extracted: E-1100

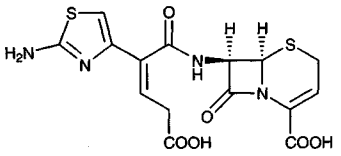
KEY WORDS

cefteram is IS; extraction of cefteram from plasma is not demonstrated

REFERENCE

Tokumura,T.; Horie,T. Determination of a novel β-lactam antibiotic (E-1100) in rat plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *620*, 153–157.

Ceftibuten



Molecular formula: $C_{15}H_{14}N_4O_6S_2$
Molecular weight: 410.43
CAS Registry No.: 97519-39-6
Merck Index: 1998
Lednicer No.: 5 160

SAMPLE

Matrix: blood
Sample preparation: Filter plasma (0.45 μm). Inject 50 μL plasma onto column A with mobile phase A, after 6 min the contents of column A were back-flushed onto column B with mobile phase B, after 3 min column A was removed from the circuit and column B was eluted with mobile phase B. Column A was washed with mobile phase C for 5 min then equilibrated with mobile phase A (1.5 mL/min) for 11 min until next injection.

HPLC VARIABLES

Column: A 35 × 4.6 20 μm TSK BSA-ODS; B 150 × 4.6 5 μm Nucleosil 5C18
Mobile phase: A MeOH:5 mM tetrabutylammonium bromide + 2 mM $(NH_4)H_2PO_4$, pH 5.0 1:50; B MeCN:MeOH:10 mM tetrabutylammonium bromide + 2 mM $(NH_4)H_2PO_4$, pH 5.0 6:3:25; C MeCN:5 mM tetrabutylammonium bromide + 10 mM $(NH_4)H_2PO_4$, pH 7.0 3:10
Flow rate: A 1.2; B 1; C 1.5
Injection volume: 50
Detector: UV 256

CHROMATOGRAM

Retention time: 22.9 (cis), 20.8 (trans)
Limit of detection: 100 ng/mL

KEY WORDS

plasma; column-switching

REFERENCE

Matsuura,A.; Nagayama,T.; Kitagawa,T. Analytical studies on β-lactam antibiotics. III. Automated high-performance liquid chromatographic method for the determination of the orally active antibiotic ceftibuten in human plasma and urine, *J.Chromatogr.*, **1989**, *494*, 231–245.

SAMPLE

Matrix: blood
Sample preparation: 50 μL Plasma + 50 μL 100 mM ammonium acetate, mix, inject a 100 μL aliquot of the supernatant onto column A with mobile phase A and elute to waste. After 4 min elute column A onto column B with mobile phase A, after 2 min elute column B with mobile phase B. At the end of each day change the guard column and wash column A with mobile phase B overnight.

HPLC VARIABLES

Column: A guard column (unspecified) + 150 × 3.9 μBondapak phenyl; B 300 × 4.6 μBondapak phenyl
Mobile phase: A 100 mM pH 6.5 ammonium acetate; B MeCN:100 mM pH 6.5 ammonium acetate 2:98
Flow rate: 1
Injection volume: 100
Detector: UV 263

CHROMATOGRAM

Retention time: 13 (cis only)

Limit of detection: 50 ng/mL
Limit of quantitation: 100 ng/mL

KEY WORDS
plasma; column-switching; pharmacokinetics

REFERENCE
Pan,H.-T.; Kumari,P.; Lim,J.; Lin,C.-C. Determination of a cephalosporin antibiotic, ceftibuten, in human plasma with column-switching high-performance liquid chromatography with ultraviolet detection, *J.Pharm.Sci.*, **1992**, *81*, 663–666.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 10 μL ceftazidime solution, homogenize, add 500 μL MeCN, vortex for 10 s, centrifuge at 3000 g for 10 min. Remove 800 μL of the supernatant and ad it to 5 mL dichloromethane, vortex for 10 s, centrifuge, inject a 50 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 20 × 4.6 10 μm Spherisorb C8
Column: 250 × 4.6 5 μm Spherisorb ODS
Mobile phase: MeCN:buffer 4.5:95.5 (Buffer was 3.85 g/L ammonium acetate + 2 mL triethylamine, adjusted to pH 4 with formic acid.)
Column temperature: 50
Flow rate: 1.75
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 7.8 (cis), 9.8 (trans)
Internal standard: ceftazidime (19.8)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Noninterfering: norfloxacin, pefloxacin, ofloxacin, amikacin, tobramycin, acyclovir

KEY WORDS
plasma; column-switching; pharmacokinetics

REFERENCE
Kinowski,J.M.; Bressolle,F.; Fabre,D.; Goncalves,F.; Rouzier-Panis,R.; Galtier,M. High-performance liquid chromatographic determination of ceftibuten and its metabolite in biological fluids: applications in pharmacokinetic studies, *J.Pharm.Sci.*, **1994**, *83*, 736–741.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 10 μL 50 μg/mL acyclovir in water + 100 μL 200 mM pH 7 sodium phosphate buffer, mix well, inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: μBondapak CN guard-PAK
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:50 mM ammonium acetate 2:98
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 11.0 (ceftibuten), 13.4 (ceftibuten-trans)
Internal standard: acyclovir (18)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, amoxicillin, ampicillin, aspirin, aztreonam, caffeine, cefamandole, cefotiam, cefsulodin, ceftazidime, ceftriaxone, cefuroxime, cephaloridine, cephalothin, chlorpheniramine, gentamicin, moxolactam, nafcillin, piperacillin, pseudoephedrine, theophylline, ticarcillin, vancomycin

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Lim,J.M.; Kim,H.; Marco,A.; Mojaverian,P.; Lin,C.-C. Liquid chromatographic determination of ceftibuten, a new oral cephalosporin, in human plasma and urine, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 699–703.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 100 μL 10 μg/mL cefadroxil + 1 mL MeCN, vortex, centrifuge at 2000 g for 10 min. Remove the aqueous phase and add it to 2.5 mL dichloromethane, vortex, centrifuge, inject a 25 μL aliquot of the upper aqueous layer.

HPLC VARIABLES
Column: 150 × 3.9 5 μm C18 (Waters)
Mobile phase: MeCN:150 mM ammonium acetate 0.7:99.3, pH 7.0
Flow rate: 1.1
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Internal standard: cefadroxil
Limit of detection: 400 ng/mL
Limit of quantitation: 500 ng/mL

KEY WORDS
serum; mouse; pharmacokinetics

REFERENCE
Onyeji,C.O.; Nicolau,D.P.; Nightingale,C.H.; Quintiliani,R. Optimal times above MICs of ceftibuten and cefaclor in experimental intra-abdominal infections, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1112–1117.

SAMPLE
Matrix: blood, dialysate, urine
Sample preparation: 100 μL Plasma, urine, or dialysate + 100 μL 200 mM pH 7 phosphate buffer + acyclovir, inject a 5 μL aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:50 mM ammonium acetate 2:98
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Internal standard: acyclovir
Limit of quantitation: 500 ng/mL (urine), 100 ng/mL (plasma)

KEY WORDS
plasma; pharmacokinetics; cis and trans isomers separated

REFERENCE
Kelloway,J.S.; Awni,W.M.; Lin,C.C.; Lim,J.; Affrime,M.B.; Keane,W.F.; Matzke,G.R.; Halstenson,C.E. Pharmacokinetics of ceftibuten-*cis* and its *trans* metabolite in healthy volunteers and in patients with chronic renal insufficiency, *Antimicrob.Agents Chemother.*, **1991**, *35*, 2267–2274.

SAMPLE
Matrix: blood, ultrafiltrate
Sample preparation: Denature with EtOH (if necessary), inject a 15 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 Nucleosil 10 C18
Mobile phase: MeCN:MeOH:PIC A 6:3:50 (PIC A is tetrabutylammonium phosphate (Waters).)
Flow rate: 1.2
Injection volume: 15
Detector: UV 256

CHROMATOGRAM
Retention time: 10.7 (cis), 12.3 (trans)
Limit of quantitation: 100 ng/mL

KEY WORDS
serum; isomerization

REFERENCE
Shimada,J.; Hori,S.; Oguma,T.; Yoshikawa,T.; Yamamoto,S.; Nishikawa,T.; Yamada,H. Effects of protein binding on the isomerization of ceftibuten, *J.Pharm.Sci.*, **1993**, *82*, 461–465.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 200 μL Plasma + 200 μL 200 mM pH 7.0 sodium phosphate, vortex, allow to sit for 15 min, add 20 μL 100 μg/mL acyclovir, add 800 μL MeCN, vortex for 20 s, centrifuge at 2500 g at 25° for 2 min. Remove the supernatant and add it to 1.6 mL dichloromethane, vortex for 20 s, centrifuge at 2500 g at 25° for 1 min, inject an aliquot of the organic layer. Urine. Dilute urine samples 10-20-fold with water, treat with Nonidet P-40 detergent, let stand for 5 min, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:50 mM ammonium acetate 2:98
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 262 (plasma), UV 254 (urine)

CHROMATOGRAM
Internal standard: acyclovir
Limit of quantitation: 500 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Kearns,G.L.; Reed,M.D.; Jacobs,R.F.; Ardite,M.; Yogev,R.D.; Blumer,J.L. Single-dose pharmacokinetics of ceftibuten (SCH 39720) in infants and children, *Antimicrob.Agents Chemother.*, **1991**, *35*, 2078–2084.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: Spherisorb 5 ODS2
Mobile phase: MeOH:10% acetic acid 25:75 or MeOH:5 mM heptanesulfonic acid 25:75
Flow rate: 1.7
Detector: UV 264

CHROMATOGRAM
Limit of detection: 250 ng/mL

KEY WORDS
pharmacokinetics; cis and trans isomers separated

REFERENCE
Wise,R.; Nye,K.; O'Neill,P.; Wostenholme,M.; Andrews,J.M. Pharmacokinetics and tissue penetration of ceftibuten, *Antimicrob.Agents Chemother.*, **1990**, *34*, 1053–1055.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 5 μm ODS Hitachi 3053
Mobile phase: MeCN:buffer 10:90 (Buffer was 50 mM citric acid and 100 mM KCl, pH 2.5.)
Flow rate: 0.7
Detector: UV 262

REFERENCE
Naasani,I.; Sugawara,M.; Kobayashi,M.; Iseki,K.; Miyazaki,K. Transport mechanism of ceftibuten, a dianionic cephem, in rat renal brush-border membrane, *Pharm.Res.*, **1995**, *12*, 605–608.

SAMPLE
Matrix: sputum
Sample preparation: Sputum + 200 μL 100 mM Ammonium acetate, vortex, centrifuge at 9000 g for 10 min, inject a 100 μL aliquot of the supernatant onto column A with mobile phase A and elute to waste. After 4 min elute column A onto column B with mobile phase A, after 2 min elute column B with mobile phase B. At the end of each day change the guard column and wash column A with mobile phase B overnight.

HPLC VARIABLES
Column: A guard column (unspecified) + 150 × 3.9 μBondapak phenyl; B 300 × 4.6 μBondapak phenyl
Mobile phase: A 100 mM ammonium acetate; B MeCN:100 mM ammonium acetate 2:98
Flow rate: 1
Injection volume: 100
Detector: MS, Vestec model 201 LC/MS, thermospray, T1 170°, probe tip 225°, block 312°, vapor 270°; monitor m/z 226

CHROMATOGRAM
Retention time: 8 (cis only, after start of elution with mobile phase B)
Limit of quantitation: 500 ng/mL

KEY WORDS
column-switching; LC-MS

REFERENCE
Pan,H.-T.; Kumari,P.; de Silva,J.A.F.; Lin,C.-C. Determination of ceftibuten in sputum by column-switching high-performance liquid chromatography on-line with thermospray mass spectrometry, *J.Pharm.Sci.*, **1993**, *82*, 52–55.

SAMPLE
Matrix: urine
Sample preparation: Dilute 1 mL urine to 10 mL with pH 7.0 phosphate buffer, filter (0.45 μm). Inject 20 μL onto column A with mobile phase A, after 1.5 min the contents of column A were forward-flushed onto column B with mobile phase B, after another 2 min column A was removed from the circuit and column B was eluted with mobile phase B. Column A was washed with mobile phase A (1.5 mL/min) for at least 10 min until next injection.

HPLC VARIABLES
Column: A 50 × 4.6 5 μm Cosmosil 5C18; B 150 × 4.6 5 μm Nucleosil 5C18
Mobile phase: A MeOH:10 mM $(NH_4)H_2PO_4$, pH 5.0 1:10; B MeCN:MeOH:5 mM tetrabutylammonium bromide + 5 mM tetraamylammonium bromide + 8 mM $(NH_4)H_2PO_4$, pH 5.0 25:10:65
Flow rate: A 1; B 1
Injection volume: 20
Detector: UV 256

CHROMATOGRAM
Retention time: 20.0 (cis), 18.6 (trans)
Limit of detection: 1000 ng/mL

KEY WORDS
column-switching

REFERENCE
Matsuura,A.; Nagayama,T.; Kitagawa,T. Analytical studies on β-lactam antibiotics. III. Automated high-performance liquid chromatographic method for the determination of the orally active antibiotic ceftibuten in human plasma and urine, *J.Chromatogr.*, **1989**, *494*, 231–245.

SAMPLE
Matrix: urine
Sample preparation: Dilute urine 1/10 to 1/40 with water, inject a 50 μL aliquot onto column A with mobile phase A, after 5 min elute the contents of column A onto column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A for 2 min.

HPLC VARIABLES
Column: A 30 × 4.6 5 μm Spheri 5 amino; B 20 × 4.6 10 μm Spherisorb C8 + 250 × 4.6 5 μm Spherisorb ODS
Mobile phase: A 30 mM NaH_2PO_4; B MeCN:buffer 2.5:97.5 (Buffer was 17.9 g/L Na_2HPO_4 adjusted to pH 7 with 10% phosphoric acid.)
Column temperature: 50
Flow rate: A 0.6; B 1

Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 6 (cis), 7 (trans)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Noninterfering: norfloxacin, pefloxacin, ofloxacin, amikacin, tobramycin, acyclovir

KEY WORDS

column-switching; pharmacokinetics

REFERENCE

Kinowski,J.M.; Bressolle,F.; Fabre,D.; Goncalves,F.; Rouzier-Panis,R.; Galtier,M. High-performance liquid chromatographic determination of ceftibuten and its metabolite in biological fluids: applications in pharmacokinetic studies, *J.Pharm.Sci.*, **1994**, *83*, 736–741.

SAMPLE

Matrix: urine
Sample preparation: Urine. 100 μL urine + 200 μL 200 mM pH 7 sodium phosphate buffer, inject a 15 μL aliquot onto column A and elute to waste with mobile phase A, after 4 min elute the contents of column A onto column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Guard column: μBondapak CN guard-PAK
Column: A 30 × 4.6 Spheri-10 amino H2GU (Brownlee); B 250 × 4.6 Partisil 10 ODS-3
Mobile phase: A 30 mM $(NH_4)H_2PO_4$; B MeCN:50 mM pH 7 sodium phosphate buffer 2.5:97.5
Flow rate: A 0.6; B 1
Injection volume: 15
Detector: UV 254

CHROMATOGRAM

Retention time: 12.3 (ceftibuten), 13.7 (ceftibuten-trans)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES

Noninterfering: aztreonam, cefmenoxime, cefoxitin, cefotiam, ceftriaxone, cephalexin

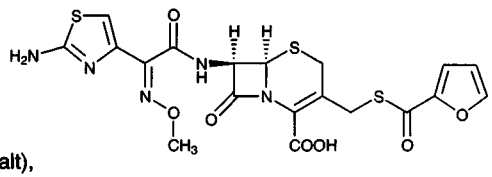
KEY WORDS

column-switching

REFERENCE

Lim,J.M.; Kim,H.; Marco,A.; Mojaverian,P.; Lin,C.-C. Liquid chromatographic determination of ceftibuten, a new oral cephalosporin, in human plasma and urine, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 699–703.

Ceftiofur



Molecular formula: $C_{19}H_{17}N_5O_7S_3$
Molecular weight: 523.57
CAS Registry No.: 80370-57-6, 104010-37-9 (sodium salt), 103980-44-5 (HCl)
Merck Index: 1999
Lednicer No.: 4 187

SAMPLE

Matrix: blood, milk
Sample preparation: 500 μL Serum or milk + 500 μL MeCN:water 50:50, vortex for 10-15 s, filter while centrifuging (Amicon Centricon-10, 10000 daltons cut-off) at 4000 g for 30 min, inject a 10-100 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 250 × 4.6 3 μm Ultremex phenyl
Mobile phase: MeCN:buffer 20:80 (Buffer was 0.25% 80% phosphoric acid, 0.25% triethylamine, 2.5 mM sodium octanesulfonate, and 2.5 mM sodium decanesulfonate in water.)
Column temperature: 40
Flow rate: 0.8-1
Injection volume: 10-100
Detector: UV 265.8

CHROMATOGRAM

Retention time: 12.5
Limit of detection: 50 ppb

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

serum; cow

REFERENCE

Tyczkowska,K.L.; Voyksner,R.D.; Anderson,K.L.; Aronson,A.L. Determination of ceftiofur and its metabolite desfuroylceftiofur in bovine serum and milk by ion-paired liquid chromatography, *J.Chromatogr.*, **1993**, *614*, 123–134.

SAMPLE

Matrix: milk
Sample preparation: Condition a 6 mL 1 g Varian Mega Bond Elut C18 SPE cartridge with 5 mL MeOH and 10 mL 100 mM ammonium acetate. Dilute 2 g milk with 8 mL 100 mM ammonium acetate, shake by hand. Add to the SPE cartridge and apply vacuum to maintain flow rate at 1-2 drops/s. Rinse the tube twice with 5 mL portions of 100 mM ammonium acetate, add the rinses to the SPE cartridge, wash with 5 mL 100 mM ammonium acetate, dry the cartridge under vacuum. Elute quickly with 3 mL MeOH. Evaporate the eluate to ca. 1 mL under a stream of nitrogen. Dilute to 2 mL with 100 mM acetate buffer. Filter (0.2 μm) and inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Supelcosil LC-18-DB
Column: 250 × 4.6 5 μm Supelcosil LC-18-DB
Mobile phase: MeCN:100 mM pH 3.3 acetate buffer 80:20 (Prepare buffer as follows. Mix 463 mL 200 mM acetic acid with 37 mL 200 mM sodium acetate and make up to 1 L with water.)

Column temperature: 35
Flow rate: 1.0
Injection volume: 100
Detector: UV 293

CHROMATOGRAM
Retention time: 12.0
Limit of detection: 4 ppb
Limit of quantitation: 7 ppb

OTHER SUBSTANCES
Simultaneous: chloramphenicol, erythromycin, gentamycin, novobiocin, penicillins, spectinomycin, sulfonamides, tetracyclines

KEY WORDS
SPE; cow

REFERENCE
McNeilly,P.J.; Reeves,V.B.; Deveau,E.J. Determination of ceftiofur in bovine milk by liquid chromatography, *J.AOAC Int.*, **1996**, *79*, 844–847.

SAMPLE
Matrix: milk
Sample preparation: Dilute 500 μL milk with 500 μL MeCN:water 50:50, vortex for 10-15 s, filter (Amicon microseparation system with 10000 molecular-mass cutoff filter (Centrion-10)) while centrifuging at 4000 g for 30 min. Inject a 100 μL aliquot of the colorless ultrafiltrate. (Protect from light!)

HPLC VARIABLES
Column: 75 × 3.9 Nova Pak C18
Mobile phase: Gradient. A was MeCN. B was 1% acetic acid containing 25 mM heptafluorobutyric acid. A:B from 5:95 to 95:5 over 9 min
Flow rate: 1
Injection volume: 100
Detector: MS, Hewlett-Packard 5989, electrospray, Hewlett-Packard API interface with hexapole ion guide, nebulizing gas nitrogen, 290°, SIM, m/z 524, post-column solvent addition of isopropanol:propionic acid 25:75

CHROMATOGRAM
Retention time: 8.7
Limit of detection: 10 ppb
Limit of quantitation: 25 ppb

KEY WORDS
ultrafiltrate; protect from light

REFERENCE
Keever,J.; Voyksner,R.D.; Tyczkowska,K.L. Quantitative determination of ceftiofur in milk by liquid chromatography--electrospray mass spectrometry, *J.Chromatogr.A*, **1998**, *794*, 57–62.

SAMPLE
Matrix: milk
Sample preparation: Mix 10 mL milk with 2 mL 100 mM tetraethylammonium chloride, add 40 mL MeCN slowly with continual stirring, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate, add 2 mL buffer, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Inject a 2 mL aliquot onto a 150 × 4.6 5 μm Supelcosil LC-18 column, elute with

MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 60:40 over 37 min at 1 mL/min, collect a 1.5-2 mL aliquot containing the compound (ca. 24.5 min), evaporate to <1 mL under reduced pressure, make up to 1 mL with water, inject an aliquot. (Prepare the buffer by mixing 10 mM KH_2PO_4 and 10 mM Na_2HPO_4 in a 5:1 ratio, pH 6.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18-DB
Mobile phase: MeCN:buffer 28:72 (Buffer was 3.3 mM phosphoric acid containing 6.7 mM potassium dihydrogen phosphate.)
Flow rate: 1
Injection volume: 200
Detector: UV 290

REFERENCE
Moats,W.A.; Romanowski,R.D. Multiresidue determination of β-lactam antibiotics in milk and tissues with the aid of high-performance liquid chromatographic fractionation for clean up, *J.Chromatogr.A*, **1998**, *812*, 237–247.

SAMPLE
Matrix: milk
Sample preparation: 10 mL Milk + 2 mL 200 mM tetraethylammonium chloride, stir, slowly add 38 mL MeCN over 30 s, let stand for 5 min, decant the supernatant through a plug of glass wool. 40 mL Filtrate + 1 mL water, evaporate under reduced pressure to 1-2 mL, make up to 4 mL with water, filter (0.45 μm polyvinylidene difluoride). Inject 2 mL into an LC system (150 × 4.6 5 μm Supelcosil LC-18; MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 40:60 over 27 min, to 0:100 over 1 min; 1 mL/min; UV 210 and 295), collect a 1.5 mL fraction at retention time for ceftiofur (23 min), evaporate to 1 mL, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18-DB
Mobile phase: MeCN:buffer 29:71 (Buffer was 3.3 mM phosphoric acid and 6.7 mM KH_2PO_4.)
Flow rate: 1
Injection volume: 200
Detector: UV 295

CHROMATOGRAM
Limit of quantitation: 2-5 ppb

OTHER SUBSTANCES
Also analyzed: ampicillin, amoxicillin, cephapirin, penicillin G, penicillin V, cloxacillin

KEY WORDS
cow

REFERENCE
Moats,W.A.; Harik-Khan,R. Liquid chromatographic determination of β-lactam antibiotics in milk: A multiresidue approach, *J.AOAC Int.*, **1995**, *78*, 49–54.

SAMPLE
Matrix: milk
Sample preparation: Condition a Bond Elut C8 SPE cartridge with 5 mL MeOH and 5 mL water. 20 mL Milk + 20 mL buffer, heat at 60° for 20 min or until milk curdles, centrifuge for 10 min, add the supernatant to the SPE cartridge, wash with two 2.5 mL portions of water, elute with 2.5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, extract the residue with three 100 μL portions of 50 mM pH 6.0

potassium phosphate buffer, filter (0.2 μm), inject an aliquot of the filtrate. (Buffer was 545 mL 100 mM citric acid, 455 mL 200 mM Na_2HPO_4, and 74.4 g EDTA, adjust to pH 4.5 with ammonium hydroxide, make up to 2 L with water.)

HPLC VARIABLES

Column: 250 × 4.6 10 μm Lichrosorb RP-8
Mobile phase: MeOH:50 mM pH 6.0 potassium phosphate buffer 35:65
Flow rate: 1
Injection volume: 200
Detector: UV 210 or Charm II assay

CHROMATOGRAM

Retention time: 18.13

OTHER SUBSTANCES

Extracted: ampicillin, cephapirin, cloxacillin, dicloxacillin, nafcillin, oxacillin, penicillin G
Simultaneous: amoxicillin

KEY WORDS

SPE

REFERENCE

Zomer,E.; Quintana,J.; Saul,S.; Charm,S.E. LC-Receptograms: A method for identification and quantitation of β-lactams in milk by liquid chromatography with microbial receptor assay, *J.AOAC Int.*, **1995**, *78*, 1165–1172.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 6 mL 1 g Mega Bond Elut C18 SPE cartridge with 4 mL MeOH and 5 mL phosphate buffer. Condition a 10 mL 500 mg Bond Elut LRC SAX SPE cartridge with 2 mL MeOH, 2 mL MeOH:100 mM NaCl 25:75, and two 1 mL portions of water. Condition a 10 mL 100 mg Bond Elut LRC SCX SPE cartridge with 1 mL MeOH, 2 mL MeOH:100 mM calcium chloride 25:75, and two 1 mL portions of water. Fat. Homogenize (Waring blender) 10 g fat, 20 mL 0.4% dithioerythritol in borate buffer, and 20 mL hexane at medium speed for 5 min, centrifuge at 3000 g for 10 min. Remove a 2 mL aliquot of the aqueous (bottom) layer and add it to 13 mL 0.4% dithioerythritol in borate buffer, shake at 50° for 15 min, add 3 mL 14% iodoacetamide in phosphate buffer, mix well, let stand at room temperature, adjust pH to 2.5-2.6 with 5% phosphoric acid, centrifuge at 4° at 48000 g for 20 min, add the supernatant to the C18 SPE cartridge, wash with 5 mL phosphate buffer, wash with 3 mL 10 mM NaOH, elute with 3 mL MeCN:water 15:85. Add the eluate to 15 mL water, add this mixture to the SAX SPE cartridge, wash with 1 mL water, elute with 2.5 mL MeCN:5% acetic acid 5:95, inject a 500 μL aliquot of the eluate. Muscle, liver kidney. Homogenize (Waring blender) 10 g tissue and 140 mL 0.4% dithioerythritol in borate buffer at medium speed for 5 min, centrifuge at 3000 g for 10 min. Remove a 15 mL aliquot of the homogenate and shake it at 50° for 15 min, add 3 mL 14% iodoacetamide in phosphate buffer, mix well, let stand at room temperature, adjust pH to 2.5-2.6 with 5% phosphoric acid, centrifuge at 4° at 48000 g for 20 min, add the supernatant to the C18 SPE cartridge, wash with 5 mL phosphate buffer, wash with 3 mL 10 mM NaOH, elute with 3 mL MeCN:water 15:85. Add the eluate to 15 mL water, add this mixture to the SAX SPE cartridge, wash with 1 mL water, elute with 2.5 mL MeCN:5% acetic acid 5:95. Add the eluate to 10 mL water, mix well, add the mixture to the SCX SPE cartridge, wash with 1 mL water, elute with 2.5 mL MeCN:100 mM NaCl 5:95 (muscle, kidney) or 2 mL MeCN:100 mM NaCl 10:90 (liver), inject a 500 μL aliquot of the eluate. (Borate buffer was 19 g sodium borate and 3.7 g KCl in 1 L water, pH 9. Phosphate buffer was 3.4 g KH_2PO_4 in 700 mL water, pH adjusted to 7 with KOH, made up to 1 L with water.)

HPLC VARIABLES

Guard column: BDS Hypersil C18
Column: 250 × 4.6 BDS Hypersil C18
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in MeCN. A:B from 100:0 to 65:35 over 35 min, wash with 50:50 at 1.5 mL/min for 15 min, re-equilibrate for 20 min (muscle, kidney) or A:B 85:15 for 5 min, to 75:25 over 5 min, wash with 50:50 at 1.5 mL/min for 15 min, re-equilibrate for 20 min (liver, fat).
Flow rate: 1
Injection volume: 500
Detector: UV 266

CHROMATOGRAM

Retention time: 7 (liver, fat), 26 (muscle, kidney)
Limit of detection: 10-30 ng/g
Limit of quantitation: 100 ng/g

OTHER SUBSTANCES

Extracted: cephapirin
Noninterfering: cefoperazone, cefquinome, cephacetril, dihydrostreptomycin, neomycin, penicillin G, spectinomycin, tetracycline

KEY WORDS

pig; muscle; kidney; liver; fat; derivatization; SPE; rugged

REFERENCE

Beconi-Barker,M.G.; Roof,R.D.; Millerioux,L.; Kausche,F.M.; Vidmar,T.J.; Smith,E.B.; Callahan,J.K.; Hubbard,V.L.; Smith,G.A.; Gilbertson,T.J. Determination of ceftiofur and its desfuroylceftiofur-related metabolites in swine tissues by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *673*, 231–244.